(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 713 701 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
29.05.1996 Bulletin 1996/22

(51) Int. Cl.[6]: **A61K 31/40**
// (A61K31/40, 31:55)

(21) Application number: **95118386.2**

(22) Date of filing: **11.05.1992**

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU MC NL PT SE

(30) Priority: **13.05.1991 IT MI911299**

(62) Application number of the earlier application in accordance with Art. 76 EPC: **92107865.5**

(71) Applicants:
- **Fraschini, Franco**
  I-20123 Milan (IT)
- **Stankov, Bojidar**
  I-20129 Milan (IT)
- **I.F.L.O. ISTITUTO FARMACOLOGICO LOMBARDO S.A.S.**
  di Giorgio & Aldo Laguzzi & C.
  I-20128 Milano (IT)

(72) Inventors:
- **Biella, Gabriele**
  I-2700 Pavia (IT)
- **Fraschini, Franco**
  I-20123 Milan (IT)
- **Stanvok, Bojidar**
  I-20129 Milan (IT)
- **Ferini Strambi, Luigi**
  I-20146 Milan (IT)

(74) Representative: **Gervasi, Gemma, Dr.**
NOTARBARTOLO & GERVASI Srl
Viale Bianca Maria 33
I-20122 Milano (IT)

Remarks:
This application was filed on 22 - 11 - 1995 as a divisional application to the application mentioned under INID code 62.

(54) 2-Bromomelatonin in the therapy of sleep disorders

(57) The present invention relates to new pharmaceutical compositions comprising therapeutically effective amounts of 2-bromomelatonin for use in the therapy of sleep disorders and in the pre-anaesthetic medication.

EP 0 713 701 A1

Description

The present invention relates to new pharmaceutical compositions comprising therapeutically effective amounts of 2-bromomelatonin, for use in the therapy of sleep disorders and in the pre-anaesthetic medication.

Melatonin is a hormone mainly sinthesized within the pineal gland, of which it is one of the most important products.

Apart the known capacity of melatonin to affect the reproductive activity in different species of mammals, the most accepted interpretation is that it is playing an essential role in the transmission of the photoperiodic information from the environment and therefore acting as a synchroniser of the endogenous biological clock.

Light is in fact the main synchroniser of the circadian and seasonal rhythms and, because of the role played by melatonin in transducing the information concerning daylength, it is accepted that it is acting as a synchroniser of the biological rythms with the day-night cycle.

Further, it is known that melatonin is controlling the sleep-wake cycle in blind people, suffering from desynchronisation of the circadian rhythm, and alleviates the problems related to rapid time zone changes (the so-called "jet-lag" which occurs following rapid transfer covering more than 5 time zones), limiting the insomnia and lethargy which follow such trips, in particular Eastward, the direction in which desynchronisation is more pronounced.

Little is still known concerning the mechanism of action of melatonin; at a molecular level its capacity to suppress the stimulation in a second messenger, namely the cAMP, has been demonstrated, but its mechanism of action at the cellular level or at the level of the neuronal circuits has not been clarified yet.

A description of the biologic activity of melatonin is reported by J. Arendt in Clinical Endocrinology (1988), 29, 205-229.

Benzodiazepines are a broad family of drugs acting on the structures of the central nervous system (CNS) through receptors of the gamma amino-butyric acid (GABA), the most widespread inhibitory neurotransmitter in CNS; they are thought to possess qualitatively similar actions, and use the same mechanisms, whereas quantitative differences may occur.

The pharmacological properties of benzodiazepines substantially spring from their action in the central nervous system. In humans, the most evident effects are sedation, sleep induction, anxiety reduction, muscle relaxation and anticonvulsive activity.

In particular all benzodiazepines express substantially similar effects on the most important sleep parameters and, with the exception of cases which require a specific therapy or non-pharmacological interventions, are considered drugs of choice for the treatment of insomnia, as they have a good therapeutical index. give rise to a smaller number of pharmacological interactions and have a low toxico-manigenic power with respect to other hypnotic drugs.

One of the drawbacks of the benzodiazepines, when used in the therapy of insomnia, is residing in that these substances do alter the compositions of the different sleep stages and the endogenous circadian rhythmicity, with consequences on the organic and psychic sphere which worsen with time.

Sleep is not a non-differentiated homogeneous process.

In fact, it consists of different stages which in normal subjects have a precise temporal organization, which has to be respected, in order to grant a sleep as natural as possible; one complete cycle of such stages requires about 90 minutes.

Sleep structure is expressed by two basic situations: the NREM sleep (non-rapid eye movements), subdivided into 4 different stages of increasing depth, and the REM sleep.

The typical sleep architecture is characterized by the recurrent, even if not necessarily hierarchical, succession of NREM stages and by fixed cycles based on the regular alternation between REM and NREM periods.

The organization of sleep into stages and cycles is supplying a structure which can fit to the variable environmental conditions.

The different stages of sleep and some of the effects coming from their lacking are reported in table 1 (from Goodman and Gilman, The pharmacological bases of the therapy, 1982, pag. 360).

Table 1

Stage	Effects coming from deprivation
0 (wake)	-
1 (benumbment, sleep is taking place)	The occurrence of the subsequent stages of the sleep is inhibited; no specific symptomatology.
2 (clear sleep condition)	The occurrence of the subsequent stages is inhinbited
3 (transition to deep sleep)	Stage 4 is inhibited
4 ("cerebral" sleep)	Suicidal ideation and diurnal terrors, remarkable rebound phenomena.
REM sleep (rapid eye movement)	Anxiety, hyperphagia, behavioural disorders; reduced learning and concentration; hypersexuality, reduced threshold for the evocation of a conclusive outline.

It is important to evaluate the sleep parameters either during the use of a drug or after its interruption, because, for instance, a too sharp suppression of stage 4 can give rise to the beginning of diurnal terrors or of suicidal ideations and to the transfer to phase (step) 2 of the nightmares usually occurring in the REM phase.

Another important parameter to evaluate, as to the sleep quality, is the intrinsic structure of the normal NREM sleep, namely the CAP (cyclic alternating pattern) consisting of periods of encephalographic activity organized in series of biphasic cycles (sleep microstructure) and the CAP-rate, defined as a per cent ratio of the CAP time with respect to the overall sleep time.

The CAP-rate parameter is thus reflecting the "sleep quality".

Benzodiazepines markedly reduce the time spent in stages 3 and 4, increase the REM latency time (the time elapsed between a sleep onset and the occurrence of the first REM episode) and moreover reduce, in general, the space occupied by the REM sleep.

It is thus clear that benzodiazepines, although increasing the depth and rest properties of the sleep and the overall length of the sleep, do not respect the physiological composition of the different sleep phases; the chronical treatment by means of such drugs can give rise to very harmful cumulative effects on the usual rythms of sleep wherefrom comes the beginning of unfavourable psychological effects, like for instance increase of the impact of the nightmares, anxiety, irritability, tachycardia, which can vary according to the employed benzodiazepine.

Further drawbacks in the benzodiazepine treatment are evident when the drug administration is interrupted after 3 or 4 weeks, and resides in a remarkable rebound effect as to the quantity and intensity of REM sleep, with reduction of the latency time and an overall increase of the time of the REM sleep, which can persist even for a long period.

Such a rebound phenomenon is corresponding to a worsened reappearance of the symptomatology preceding the pharmacological treatment, namely reappearance of anxiety, of insomnia and so on.

At last another undesired effect of benzodiazepines is related to the drug administration in a high dosage or for a prolonged time and resides in the development of tolerance and addiction.

These drugs become ineffective when administered for a long time and therefore higher dosages are required in order to achieve the same effects.

The development of tolerance and rebound effects provide anxiety and usually lead to the need of higher and more frequent dosages of drug, thus creating a vicious circle between dosage increase and tolerance, with the conclusive consequence of an alteration of the normal rhythm of the sleep.

The pharmaceutical compositions comprising therapeutical amounts of 2-bromomelatonin are new.

The pharmaceutical compositions according to the present invention allow for better results in the treatment of the sleep disorders and in the pre-anaesthetic medication.

The patients taking the pharmaceutical compositions according to the present invention, show a better synchronisation of the diurnal activity, a reduced sleep latency and a longer sleep period; the general conditions, subjectively evaluated by each person, and the "performance status" are considerably improved.

Melatonin and its derivatives are further completely devoid of toxicity, even in higher doses, with respect to the dosage to be used in the therapy of sleep disorders.

The pharmaceutical compositions according to the present invention comprise 2-bromo-N-acetyl-5-methoxytriptamine (2-bromo-melatonin), at a dosage comprised between 10 and 40 mg.

These pharmaceutical compositions can be administered orally or parenterally and, depending on the desired pharmaceutical form, they will contain all the normally required excipients, commonly used in the pharmacological practice.

The pharmaceutical compositions according to the present invention have to be administered 30-40 minutes before going to bed or before induction of anaesthesia.

In order to show the activity of the compositions according to the present invention, we carried out several tests, taking into consideration different physiological parameters, like, for instance, the data obtained by means of electrocardiogram (ECG) and electroencephalogram (EEG), breathing frequency, objective analysis and personal evaluation.

For exemplification purposes, we report the results of some of the series of performed tests.

Series I

We evaluated the effects of melatonin, 2-iodomelatonin and 2-bromomelatonin on the spontaneous firing activity of single neurons of the parietal cortex of rabbit and of the thalamus nuclei of rat, where we have previously found and described the presence of melatonin receptors.

Materials and methods

Our experimental work was carried out by means of electrophysiological techniques using extracellular registration of single neurons (thalamic or cortical neurons), of the tested animal species (rat and rabbit) anaesthetized by means of urethane or barbiturates. Such recordings were effectuated before, during and after microionophoretic or micropressive application of the substances: by means of multichannel glass microelectrodes. The concentration of the used substances was respectively: Melatonin, 2-bromomelatonin or 2-iodomelatonin (10^{-6}-10^{-7}M), GABA 10^{-2}M, Bicuculline 10^{-4}M. The neurons sensitive to GABA (90% of the studied ones) showed powerful inhibitions when subjected to the GABA action.

The basal neuronal activity was taken as 100% and the difference in activity obtained following administration of the tested compounds was evaluated as per cent of the basal activity.

The results are reported in Table 2.

Table 2

Active Principle	Administration time (min)	Duration of the effect post-administration (min)	Activity after administration (%basal activity)
Melatonin (100 nM)	5	2.5	87
Melatonin (1 μM)	5	4	56
GABA (1 mM)	0.1	0.01	34
GABA (1 mM) +	0.1		
Melatonin (1 μM)	2	6	20
2-iodomelatonin (100 nM)	5	5	47
GABA (1 mM) +	0.1		
2-iodomelatonin (100 nM)	2	7	10
2-bromomelatonin (100 nM)	5	10	14
GABA (1 mM) +	0.1		
2-bromomelatonin (100 nM)	2	13	11

* basal activity = 100 %

As it clearly results from the data reported in table 2, the administration into the parietal cortex of amounts of the order of nanograms of 2-iodomelatonin and 2-bromomelatonin gave rise to long lasting, powerful inhibitions of the spontaneous activity of both cortical and thalamic neurons.

The administration of melatonin alone, in the same concentration range, gave rise to analogous, though less marked effects, whereas the administration of melatonin and GABA resulted in a considerable inhibition of the spontaneous activity.

GABA is the most common inhibitory neuro transmitter in the central nervous system. The GABA-receptor consists of a macromolecular complex which, besides the sites having strong affinity to the specific transmitter, namely to GABA, possess "modulation" sites where many different substances and molecules are acting.

In general, it is required that the receptor is activated by a previously bound transmitter. Such activation is followed by a series of phenomena which facilitate the binding of the modulating substances. Some molecules, like the ones object of the present invention, as the melatonin halogenated compounds, are showing an "autonomous" (independent) capacity to activate the GABA receptor, which is evident either when the substance is administered alone or in association with GABA itself, whose inhibitory transmitter properties are dramatically augmented by said molecules.

Melatonin, on the contrary, like the benzodiazepines, requires receptor preactivation on the part of GABA.

Either the autonomous mechanisms or the ones dependent on GABA give rise to effects similar to the benzodiazepinic ones, which can be exploited either indipendently or in association with the benzodiazepines themselves.

Series II

It is well known that the action of benzodiazepines on healthy subjects is limited and we tested therefore the activity of melatonin agonists in patients suffering from insomnia.

We carried out another series of trials, determining the effects of 2-bromomelatonin administration (2-BrMel) and Triazolam on the sleep parameters in patients suffering from psycho-physiologic insomnia. Twelve patients were successively treated with 2-bromomelatonin (10 mg per os) or Triazolam (0.125 mg per os).

Materials and methods

All patients were uniformely selected and classified as suffering from psychophysiological insomnia, according to "The International Classification of Sleep Disorders; Diagnostic and Coding Manual, ASDA, 1990".

The substances were administered at 22.30 h and the polyinsomnographic recording was performed from 23.00 to 07.00 h, by using Medilog 9000 with 8 channel recorder.

The results of this double blind test are reported in table 3.

Table 3

	Baseline	2-BrMel	TRI
Sleep macrostructure			
SL (min)	20.2	19.0	19.7
WASO (min)	61.1	42.0*	51.2*
SE (%)	83.5	87.0*	81.6
WASO (n°)	15.5	11.0*	11.7*
Sleep architecture			
St. 1 NREM (%)	6.8	6.9	5.8
St. 2 NREM (%)	54.3	54.8	54.3
St. 3-4 NREM (%)	16.6	16.8	16.6
REM (%)	21.6	22.7	22.8
REM Lat. (min)	79.8	86.2	85.5
REM period (n°)	4.8	4.2	5.0
St. shifts/h (n°)	9.0	8.1	8.0
Sleep microstructure			
CAP rate (%)	43.0	32.0*	28.2*

* $p < 0.05$ vs Baseline (Dunnett)

These data clearly show that the administration of only 10 mg of 2-bromomelatonin resulted in significant changes in the basic sleep parameters, that were even more pronounced when compared with the administration of 0.125 mg of Triazolam.

In particular, 2-bromomelatonin proved to be more effective than Triazolam regarding some parameters, such as, for instance, "sleep efficiency" and WASO.

A very favourable result emerging from these experimental trials is that the sleep architecture was not altered, whereas the efficiency and the quality of sleep were considerably improved.

For illustrative and not limiting purposes, we report pharmaceutical compositions according to the present invention.

Example 1

2-bromomelatonin containing capsule
2-bromomelatonin 10 mg
fructose 20 mg

Example 2

Soft gelatine capsule
2-bromomelatonin 10 mg
polyethylene glycol 400 200 µl (50% solution)

Experimental data

We carried out further studies on healthy volunteers and on patients suffering from psychophysiological insomnia; the results showed the evidence of an unexpected activity.

A double-blind, placebo-controlled approach was adopted, for all the experiments and the sleep parameters were evaluated by a "blind" evaluator. All participants gave informed consent to be enrolled in the studies, and the studies design, the protocols and the methods for evaluation were approved by the Institutional Ethics Committee.

Study 1

The laboratory data in animal models obtained by in situ iontophoretic drug administration, suggested that some melatonin agonists, (e.g. 2-bromomelatonin) possess similar intrinsic activities, that do not require concomitant activation of the gamma-aminobutyric acid (GABA - receptor) complex by exogenously applied GABA, as is the case with melatonin.

The possibility that 2-bromomelatonin may positively influence sleep in patients suffering from psychophysiological insomnia was investigated.

Twelve male patients (46 ± 8.6 years) were successively treated with melatonin (10 mg), 2-bromomelatonin (10 mg), triazolam (0.125 mg) or placebo. All patients were uniformly selected and classified as suffering from psychophysiological insomnia, according to The International Classification of Sleep Disorders: Diagnostic and Coding Manual, ASDA, 1990.

The drugs were administered orally in color-coded gelatine capsules at 22:30 h. The lights were turned off at 23:00 h and the polysomnographic recordings started and obtained until 07:00 h, the following morning.

The protocol was random, and the study design was double-blind, placebo-controlled. At the end of the experiment, the sleep score was evaluated, the code was broken, and the results evaluated.

RESULTS, Study 1

TABLE I

SLEEP INITIATION AND MAINTENANCE				
	Baseline	aMT 10 mg	TRI 0.125 mg	2-BrMel 10 mg
TST (min)	449.8 ± 32.2	447.2 ± 31.8	470.0 ± 49.1	486.2 ± 29.5
SL (min)	20.2 ± 16.8	21.0 ± 12.6	17.7 ± 15.2	19.0 ± 11.9
WASO (min)	61.1 ± 25.4	62.6 ± 23.6	51.2 ± 27.2	42.0* ± 17.9
Awakenings (n)	15.5 ± 3.7	16.1 ± 3.9	11.7* ± 4.6	11.0* ± 3.7
SE (%)	83.5 ± 4.4	82.9 ± 3.0	81.6 ± 3.0	87.0* ± 3.2
Values are expressed as mean ± SD TST = Total Sleep Time; WASO = Wake After Sleep Onset; SE = Sleep Efficiency				

* $p<0.05$ vs Baseline and aMT.

TABLE II

SLEEP ARCHITECTURE				
	Baseline	aMT 10 mg	TRI 0.125 mg	2-BrMel 10 mg
St.1 NREM (%)	6.8 ± 2.6	6.7 ± 2.5	5.8 ± 1.9	6.9 ± 3.6
St.2 NREM (%)	54.3 ± 6.0	54.1 ± 6.9	54.3 ± 3.3	54.8 ± 9.6
St.3-4 NREM (%)	16.6 ± 8.0	16.0 ± 7.4	16.6 ± 3.2	16.8 ± 2.9
REM (%)	21.6 ± 4.3	22.1 ± 5.1	22.8 ± 4.4	22.7 ± 6.3
REM latency (min)	79.8 ± 22.5	81.6 ± 26.4	85.5 ± 28.8	86.2 ± 38.4
REM periods (n)	4.8 ± 0.9	4.6 ± 0.8	5.0 ± 0.8	4.2 ± 1.0
Stage shifts/h (n)	9.0 ± 1.9	8.9 ± 1.8	8.0 ± 2.1	8.1 ± 2.0
Value are expressed as mean ± SD.				

From the macrostructure data, surprisingly an extremely low dose of 2-bromomelatonin (10 mg) showed effects very similar to those of a standard dose of triazolam (0.125 mg), and in the case of sleep efficiency 2-bromomelatonin was significantly better than triazolam.

On the contrary, melatonin in the same dose (10 mg) was without any effect. The evaluation of the sleep microstructure extended the results regarding the efficacy of 2-bromomelatonin.

TABLE III

MICROSTRUCTURE OF SLEEP				
	Baseline	aMT 10 mg	TRI 0.125 mg	2-BrMel 10 mg
CAP cycle (n)	246.3 ± 46.1	238.1 ± 45.8	163.5* ± 37.4	188.2* ± 40.8
CAP rate	43.0 ± 6.9	45.3 ± 7.6	28.2* ± 6.5	32.0* ± 7.1
Values are expressed as mean ± SD				

* $p<0.05$ vs Baseline and aMT

The number of CAP cycles and the CAP rate were higher in this sample of patients suffering from insomnia and Melatonin, in a dose of 10 mg was without any effect.

2-Bromomelatonin significantly reduced the number of CAP cycles and the CAP rate, compared to baseline ($p<0.05$), and its effect was not different from that of a single standard triazolam dose.

The data demonstrate that 2-bromomelatonin surprisingly expressed clear benzodiazepine-like properties in a very low dose of 10 mg.

The result was completely unpredictable because native melatonin at the same dose was inactive in regard to hypnotic activity.

The peripheral blood half-life of 2-bromomelatonin is relatively short (≈ 60 minutes) and 2-bromomelatonin is virtually devoid of acute toxicity (ED_{50} ≈ 800 mg/kg, orally in mice, and >1000 mg/kg orally in the rat).

Claims

1. Pharmaceutical compositions comprising therapeutically effective amounts of 2-bromomelatonin for use in the therapy of sleep disorders and in the pre-anaesthetic medication.

2. Pharmaceutical compositions according to claim 1, characterized in that the therapeutically effective amount is comprised between 10 and 40 mg.

3. Use of 2-bromomelatonin in association with a centrally acting benzodiazepine for the preparation of pharmaceutical compositions having an activity in the therapy of sleep disorders and in the preanaesthetic medication.

4. Use according to claim 3, characterized in that the therapeutically effective amount is comprised between 10 and 40 mg..

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 11 8386

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X	J. PINEAL RES., vol. 7, no. 2, 1989 pages 205-209, SUGDEN ET AL. 'Antigonadal activity of the melatonin analogs 2-iodomelatonin and 2-chloromelatonin in the juvenile Djungarian hamster , Phodopus sungorus campbelli' * the whole document *	1	A61K31/40 //(A61K31/40, A61K31:55)
A	---	3	
A	LIFE SCI., vol. 40, no. 15, 1987 pages 1537-1543, SURANYI-CADOTTE ET AL. 'Coexistence of central and peripheral benzodiazepine binding sites in the human pineal gland' * the whole document *	3	

A	BIOCHEM. PHARMACOL., vol. 40, no. 12, 1990 pages 2701-2705, NILES ET AL. 'Pharmacological inhibition of forskolin-stimulated adenylate cyclase activity in rat brain by melatonin, its analogs and diazepam' * the whole document *	3	TECHNICAL FIELDS SEARCHED (Int.Cl.5) A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 February 1996	Gac, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)